Sequence Listing in full is provided on disk. The content of the computer readable copy of the Sequence Listing is the same as the enclosed paper copy, and adds to the Sequence Listing only sequences previously disclosed on page 56, line 14 of the specification as originally filed. The objection to claim 37 under 37 C.F.R. § 1.821(d) is believed to be rendered moot by the amendment of claim 37 and of the Sequence Listing; thus, withdrawal of the objection is requested.

Claims 21-42 stand rejected under 35 U.S.C. §103(a) as allegedly obvious based on Chaudhary et al., P.N.A.S. 37: 9491-9494 (1990), in view of Neville et al., P.N.A.S. 89:2585-2589 (1992), Hirsch et al., Transplantation 49: 1117-1123 (1990), and Whitlow et al., Methods: A Companion to Methods in Enzymology 2:97-105 (1991). The Office Action states that Chaudhary et al. teaches how to make a DT388-anti-Tac (Fv) construct but concedes that it does not teach the DT 390 toxin moiety and does not teach an anti-CD3 antibody moiety. The Office Action further cites Neville et al. for teaching the use of immunoconjugates, comprising a toxin moiety and a whole anti-CD3 antibody. The Office Action also cites Hirsch et al. for teaching the use of the anti-CD3 $F(ab')_2$ fragment in immunosuppression and inhibition of skin graft rejection. Hirsch et al., however, fails to teach either a fusion protein or a molecule further comprising a toxin moiety. Finally, the Office Action cites Whitlow et al. for teaching fusion proteins containing an engineered single chain Fv, including a linker. Applicants respectfully

assert that the cited references do not render the claimed invention obvious and traverse the rejection.

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there *must* be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there *must* be a reasonable expectation of success. Finally, the prior art reference (or references when combined) *must* teach or suggest all the claim limitations.
>
> The teaching or suggestion to make the claimed combination and the reasonable expectation of success *must* be found in the prior art, not in the applicant's disclosure.

See MPEP § 2143 (emphasis added).

The Examiner does not identify support in the references themselves to suggest the combination of the references but, nonetheless, asserts that one skilled in the art would have been motivated to combine the teachings of all four references "with a reasonable expectation of success . . . based on the fact that one would want to more specifically target the cells responsible for the etiology of a subject's condition." See Office Action, page 4, line 26. In Chaudhary et al., page 9494, first paragraph of Discussion section, however, the authors caution that the toxicity of DT388-anti-Tac (Fv) construct was not tested in subjects, merely in cell lines.

Furthermore, Whitlow et al., page 98, first column, specifically states that "[t]o date, no report of an sFv fusion protein being tested *in vivo* has been published." In the absence of any support in the cited references for *in vivo* data for *any* fusion protein, an assertion that a single chain variable region of an anti-CD3 antibody linked to a toxin moiety would have the desired toxic and targeting effects to treat a subject's condition employs hindsight. Such hindsight is impermissible. See M.P.E.P. § 2142 ("[T]he examiner must step back in time and into the shoes worn by the hypothetical 'person of ordinary skill in the art' when the invention was unknown and just before it was made."). *See also In re Fritch*, 972 F.2d 1260 (Fed. Cir. 1992) ("Here, the Examiner relied upon hindsight to arrive at the determination of obviousness. It is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious."). There is no suggestion or motivation in the art to arrive at the claimed compositions.

Furthermore, one of ordinary skill in the art would not have had a reasonable expectation of success. It is impossible to know *a priori* the efficacy of a specific fusion immunotoxin that utilizes an sFv as a binding moiety even if the relative affinity of the parental antibody is known or the toxicity of a chemical immunoconjugate using the parental antibody is known. This is because sFv moieties are monovalent whereas the parental antibodies are divalent. The bioactivity may depend on divalent binding. This may be solely related to a higher affinity. Alternatively it may be related to the cross-linking of two adjacent epitopes which promotes

fusion toxin uptake and translocation to the cytosol compartment. It is unpredictable whether the sFv coupled with a toxin moiety will have lower bioactivity as "the monovalent binding of the sFvs may limit their effective use." See Owens & Young, *The Genetic Engineering of Monoclonal Antibodies*, J. Immunol. Methods 168:149-65, 156) (attached). Thus, the cited references, at best, suggest that it might be obvious to try to create a useful fusion immunotoxin comprising a single chain variable region of an anti-CD3 antibody linked to a toxin moiety. The court in *In re Geiger,* 815 F.2d 686, 688 (Fed .Cir. 1987), held that even though "one skilled in the art might find it obvious to try various combinations" this does not meet the burden imposed on the PTO. In the present case, one skilled in the art would have a reasonable basis to expect a fusion immunotoxin comprising a single chain variable region of an anti-CD3 antibody linked to a toxin moiety would lack bioactivity. It was only after construction and testing of the fusion immunotoxin that the bioactivity could be ascertained. Applicants respectfully assert that the rejection impermissibly employs hindsight based on the applicant's own specification and is therefore improper.

The Examiner further cites Youle et al., J. Immunology 136:93-98, to support his rejection under § 103. Youle et al., however, fails to teach an immuntoxin fusion protein, and fails to teach the use of a single chain variable region antibody moiety, and fails to teach a modified toxin moiety. Nothing in this additional reference suggests either the combination of references or a reasonable likelihood of success. Thus, applicants assert that Youle et al., in

combination with the other cited references, does not render the claimed invention obvious. Applicants thus request reconsideration and withdrawal of the rejection.

No new matter is believed to be added by any of the preceding amendments. Thus, consideration and allowance of the pending application are respectfully requested. The Examiner is invited to contact the undersigned counsel by telephone if such contact would expedite prosecution.

A check in the amount of $290.00 is enclosed with a Request for an Extension of Time and Supplemental Information Disclosure Statement. This amount is believed to be correct; however, the Commissioner is hereby authorized to change any additional fees that may be required or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Box Fee Amendment, Assistant Commissioner of Patents, Washington, D.C. 20231, on the date indicated below.

Tina Williams McKeon — April 3, 2001 (Date)

EXHIBIT A

MARKED-UP VERSION
AMENDMENTS TO THE SPECIFICATION

Page 1 (previously amended by the Continuing Application Transmittal Form):

This application is a continuation of, and claims the benefit of, Serial No. 08/739,703, filed October 29, 1996, which status is [pending] abandoned, and which application is hereby incorporated herein by reference, and claims the benefit of priority of provisional application Serial No. 60/008,104, filed October 30, 1995.

Page 41 (previously amended by preliminary amendment):

The recombinant immunotoxin, sFv-DT390, was generated in two phases. First the coding sequences for the variable light (V_L) and variable heavy (V_H) chain regions of the UCHT1 antibody were amplified by a two step protocol of RT-PCR using primers based on the published sequence (13). The 5′ V_L primer added a unique NcoI restriction enzyme site while the 3′ V_H primer added a termination codon at the J to constant region junction and an EcoRI site. The V_L region was joined to the V_H region by single-stranded overlap extension and the two regions are separated by a $(Gly_4Ser)_3$ (SEQ ID NO:15) linker that should allow for proper folding of the individual variable domains to form a function antibody binding site (14). Second, genomic DNA was isolated from a strain of *C. diphtheriae* producing the DT mutant CRM9 (C7[$\beta^{tox\text{-}201tox\text{-}9h'}$]) as described (15). This DNA was used for PCR. The 5′ primer was specific for the toxin gene beginning at the signal sequence and added a unique NdeI restriction site. The 3′ primer was specific for the DT sequence terminating at amino acid 390 and added an NcoI site in frame with the coding sequence. The PCR products were digested with the appropriate restriction enzymes and cloned into the *E. coli* expression plasmid pET-17b (Novagen, Inc., Madison, WI, USA) which had been linearized with NdeI and EcoRI. The resulting plasmid was used to transformed *E. coli* BL21/DE3 cells. Cells were grown to an OD_{590} of 0.5, induced with 0.5 M IPTG (Invitrogen, San Diego, CA, USA) and incubated for an additional 3 hours. The sFv-DT390 protein was isolated in the soluble fraction after cells were broken with a French Press and the lysate subjected to centrifugation at 35,000 X g.

Page 49 (previously amended by preliminary amendment):

Having observed that the epitope(s) recognized by the antibodies important for protection lay in the C-terminal 150 amino acids, a single-chain immunotoxin was generated with the first 390 amino acids (out of 535) of DT. Position 390 was chosen for 2 reasons: first, the 3 dimensional structure of DT suggested that this position was an external point on the molecule away from the enzymatic domain (18), and second, fusion toxins have been generated with longer DT subfragments with no reports of serum effects (19). The DNA encoding the first 390 amino acids of DT was ligated to DNA encoding the anti-CD3εsFv (V_L linked to V_H using a $(Gly_4Ser)_3$ (SEQ ID NO:15) linker sequence). The predicted molecular weight for the fusion protein is 71,000 Daltons and has been confirmed by Western Blot analysis of both *in vitro* transcribed and translated protein as well as protein isolated from *E. coli* using goat anti-DT antibodies. The toxicity of sFv-DT390 protein, isolated from *E. coli* strain BL21/DE3, was compared to UCHT1-CRM9 in protein synthesis inhibition assays (Figure 3A). The IC_{50} (concentration required to inhibit protein synthesis to 50% of controls) of sFv-DT390 was 4.8×10^{-11} M compared to 2.9×10^{-12} M for UCHT1-CRM9, a 16-fold difference. To demonstrate the specificity of the sFv-DT390 construct, competition experiments were performed using increasing concentrations of UCHT1 antibody as competitor (Figure 3B). The results showed that approximately 1/8 antibody is needed to compete the sFv-DT390 toxicity to 50% as compared to UCHT1-CRM9. The antibody was capable of totally competing toxicity of both constructs thereby showing their specificity. The immunotoxins were then subjected to protein synthesis assays in the presence of increasing dilutions of serum (Table 5).

Page 56 (amended):

Primers used for the antibody engineering are listed in Table 6, and the primer sequences are based on published data [13]. The procedures of cloning scUCHT1 is schematically depicted in Fig. 4. mRNA isolated from UCHT1 hybridoma cells (provided by Dr. P. C. Beverley, Imperial Cancer Research Fund,London was reverse transcribed into cDNA. The V_L and V_H regions of UCHT1 were amplified with polymerase chain reaction (PCR) from the cDNA usingprimer pairs P1, P2 and P3, P4 respectively. Primers P2 and P3 have a 25 bp complementary overlap and each encoded a part of a linker peptide $(Gly_4Ser)_3$ (SEQ ID NO:15). The single chain variable fragment (V_L-linker-V_H) was created by recombinant amplification of V_L and V_H using primers P1 and P4. A mouse kappa chain signal sequence was added at the V_L 5'-end by PCR, first with primers SP2 and P4, and then with primers SP1 and P4. The human IgM Fc region (CH_2 to CH_4) was amplified from the plasmid pBlue-huIgM (kindly provided by Dr. S. V. S. Kashmiri, National Cancer Institute, Bethesda. This gene fragment was

about 1.8 kb. The V_L-linker-$_{VH}$-CH2 region which is important for antigen recognition was confirmed by sequence analysis. Finally, the single chain variable fragment and the human IgM Fc region were cloned into plasmid pBK/CMV (Stratagene, La Jolla, CA, USA). Using the generated pBK/scUCHT1 plasmid as template, an *in vitro* transcription/translation assay yielded a product of 75 kDa, the expected size.

AMENDED CLAIMS

37. (Amended) A fusion immunotoxin, consisting of DT390 linked via its carboxy terminus through a linker to the variable light domain of UCHT1 which is linked via its carboxy terminus through a $(Gly_4Ser)_3$ (SEQ ID NO:15) linker to the variable heavy domain of UCHT1.